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Microvascular oxygen pressures in muscles comprised of different fiber types: Impact of dietary nitrate supplementation

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ABSTRACT

Nitrate (NO_3^-) supplementation via beetroot juice (BR) preferentially improves vascular conductance and O_2 delivery to contracting skeletal muscles comprised predominantly of type IIb + d/x (i.e. highly glycolytic) fibers following its reduction to nitrite and nitric oxide (NO). To address the mechanistic basis for NO_3^- to improve metabolic control we tested the hypothesis that BR supplementation would elevate microvascular PO_2 (PO_2mv) in fast twitch but not slow twitch muscle. Twelve young adult male Sprague–Dawley rats were administered BR ([NO_3^-] 1 mmol/kg/day, $n = 6$) or water (control, $n = 6$) for 5 days. PO_2mv (phosphorescence quenching) was measured at rest and during 180 s of electrically-induced 1-Hz twitch contractions (6–8 V) of the soleus (9% type IIb +d/x) and mixed portion of the gastrocnemius (MG, 91% type IIb + d/x) muscles. In the MG, but not the soleus, BR elevated contracting steady state PO_2mv by ~43% (control: 14 ± 1, BR: 19 ± 2 mmHg ($P < 0.05$)). This higher PO_2mv represents a greater blood–myocyte O_2 driving force during muscle contractions thus providing a potential mechanism by which NO_3^- supplementation via BR improves metabolic control in fast twitch muscle. Recruitment of higher order type II muscle fibers is thought to play a role in the development of the $\dot{V}O_2$ slow component which is inextricably linked to the fatigue process. These data therefore provide a putative mechanism for the BR-induced improvements in high-intensity exercise performance seen in humans.

1. Introduction

At exercise onset, the immediate increase in ATP turnover within contracting skeletal muscle mandates an elevated rate of O_2 delivery ($\dot{Q}O_2$), such that capillary blood flow is rapidly increased to meet the rising O_2 demand of contracting myocytes (i.e. O_2 uptake; $\dot{V}O_2$) [1]. This augmented capillary flow is accomplished via elevated cardiac output and blood flow redistribution (neurohumoral activation) as well as local mechanical and vasomotor mechanisms (reviewed in Ref. 2). Of the local controllers, the powerful signaling molecule nitric oxide (NO) promotes vasodilation of arterioles within skeletal muscle, helping to facilitate this hyperemic response and better match $\dot{Q}O_2$ to the elevated $\dot{V}O_2$ demands [3–5].

It is now understood that nitrate (NO_3^-) and nitrite (NO_2^-) can be converted to NO and other reactive nitrogen species in vivo following a stepwise reduction (reviewed in Ref. 6). In humans, dietary NO_3^- supplementation has been shown to enhance muscle contractile [7] and mitochondrial (i.e. force or work/ $\dot{V}O_2$ and P/O ratio respectively; [8]) efficiency, both of which are associated with a reduction in the O_2 cost of submaximal exercise [7–14] and improvements in tolerance to high intensity exercise [7,9,11,13,15–18].

What is particularly interesting is that the improvements in performance have been seen predominantly during severe-intensity exercise [15,19] rather than long term endurance exercise [20,21]. Recent studies performed in murine models suggest that this phenomenon may be due to a fiber type selective enhancement in skeletal muscle vascular and metabolic control following NO_3^- ingestion [22,23]. Specifically, our laboratory has demonstrated that rats supplemented with beetroot juice (NO_3^- concentration 1 mmol/kg/day) for 5 days had higher exercising blood flow and vascular conductance in muscles comprised principally of type II muscle fibers [22]. BR also raised the pressure head for capillary–myocyte O_2 flux during the crucial transition period from rest to muscle contractions (i.e., ~20–60 s) in the rat spinotrapezius muscle, which is composed of approximately 50% type IIb + d/x muscle fibers [24,25]. Moreover, Hernandez et al. [23] demonstrated improved calcium

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handling and rate of force development in type II but not type I muscles of NO_3^- supplemented mice.

Given the lower contracting PO_2mv reported in fast twitch muscles [26] and the evidence that NO_2^- reduction to NO is potentiated in environments with low PO_2 [27] the physiological effects of BR on the PO_2mv profile may be intensified in fast twitch muscles. Therefore the purpose of the present investigation was to examine the effects of 5 days of NO_3^- supplementation via BR (NO_3^- concentration 1 mmol/kg/day) on the PO_2mv profile of rat muscles comprised of predominantly type I (slow twitch) and type IIb + d/x (fast twitch) muscle fibers. We tested the hypothesis that BR would attenuate the fall in PO_2mv in the fast twitch mixed portion of the gastrocnemius (MG) across the rest–contraction transition with either a lesser or no effect in the slow twitch soleus muscle.

2. Methods

2.1. Animal selection and care

Twelve young adult male Sprague–Dawley rats (average body mass = 521 ± 20 g, Charles River Laboratories, Wilmington, MA) were used in this investigation. Rats were maintained in accredited animal facilities at Kansas State University on a 12/12 h light–dark cycle with food and water provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University and conducted according to National Institutes of Health guidelines.

2.2. Supplementation protocol

Rats were randomly assigned to receive 5 days of BR supplementation with a NO_3^- dose of 1 mmol/kg/day (BR; $n = 6$, Beet it™, James White Drinks, Ipswich UK, diluted with 100 ml of tap water) or NO_3^- depleted BR (control; $n = 6$, Beet it™ placebo, diluted with 100 ml of tap water) with consumption monitored. This NO_3^- dose (1 mmol/kg/day) represents a NO_3^- concentration similar to that used in humans by Jones and colleagues [9,11,14,18] after accounting for the resting metabolic rate of rats (approximately seven times that of humans [28]). In addition, this dose was used in our laboratory previously with significant vascular effects observed following supplementation [22,25].

2.3. Surgical instrumentation

Rats were anaesthetized with a 5% isoflurane–O_2 mixture and maintained subsequently on 3% isoflurane–O_2 mixture. The carotid artery was cannulated and a catheter (PE-10 connected to PE-50, Intra-Medic polyethylene tubing, Clay Adams Brand, Becton, Dickinson and Company, Sparks, MD) inserted into carotid artery for measurement of mean arterial pressure (MAP) and heart rate (HR), arterial blood sampling (Nova State Profile M, Waltham, MA, USA) and infusion of the phosphorescent probe (see discussion later). A second catheter was also placed in the caudal artery. Incisions were then closed and the rats were transitioned to pentobarbital sodium anesthesia (administered to effect and subsequently maintained via the caudal artery catheter) with level of anesthesia monitored continuously via the toe pinch and blink reflexes. If indicated, additional pentobarbital sodium was administered in supplemental dosage (5–10 mg/kg) as needed. Rats were then transferred onto a heating pad to maintain core body temperature at ~38 °C (measured via rectal probe thermometer) and the carotid artery catheter was connected to a pressure transducer (Digi-Med BPA model 200, Louisville, KY, USA) for measurement of MAP and HR.

The muscles chosen for the present experiment (soleus and mixed portion of the gastrocnemius, MG) were selected based on their fiber type composition [24] and represent the spectrum of slow twitch (type I/IIa) and fast twitch (type IIb + d/x) muscle fiber types. The highly oxidative soleus (84% type I, 7% type IIa and 9% type IIb + d/x [24]) serves as a postural muscle whose primary functions are ankle stabilization and plantar flexion while the MG functions in plantar flexion and is comprised predominantly of highly glycolytic fast-twitch muscle fibers (3% type I, 6% type IIa, 91% type IIb + d/x [24]). Each muscle was exposed for PO_2mv experiments in the following manner. Overlaying skin and fascia along the sagittal plane on the right hindlimb were reflected carefully to expose the muscles of the 'calf'. For measurements made in the MG, silver wire electrodes were sutured (6-0 silk) to the proximal (cathode) and distal (anode) portions of the muscle. Following measurements of PO_2mv in the MG the soleus muscle was exposed by carefully reflecting overlaying tissue covering the peroneal muscle group (coronal plane) and silver wire electrodes were sutured (6-0 silk) in the same manner as for the MG. Measurement order was randomized to avoid the potential influences of an ordering effect caused by NO_3^- metabolism. The exposed muscles were continuously superfused with warmed (38 °C) Krebs–Henseleit bicarbonate buffered solution equilibrated with 5% CO_2–95% N_2 and surrounding exposed tissue was covered with Saran wrap (Dow Brands, Indianapolis, IN, USA). This method has been used previously in our laboratory and facilitates access to the MG and soleus muscles while minimizing perturbation caused by surgery [29].

2.4. Experimental protocol

The phosphorescent probe palladium meso-tetra (4 carboxyphenyl)tetrabenzoporphyrin-dendrimer (G2: 1–5 mg/kg dissolved in 0.4 ml saline) was infused via the carotid artery catheter. After a brief stabilization period (~10 min), the common end of the light guide of a frequency domain phosphorimeter (PMOD 5000, Oxygen Enterprises, Philadelphia, PA, USA) was positioned ~2–4 mm superficial to the lateral surface of the exposed muscle (either MG or soleus) of the right hindlimb over a randomly selected muscle field absent of large vessels thus ensuring that the region contained principally capillary blood. PO_2mv was measured via phosphorescence quenching (see discussion below) and reported at 2 s intervals throughout the duration of the 180 s contraction protocol (1 Hz, ~6 V, 2 ms pulse duration) elicited via a Grass stimulator (model S88, Quincy, MA, USA). As an indicator of preserved vasomotor function, it was ensured that PO_2mv returned to baseline values following the contraction period. Rats were euthanized via pentobarbital sodium overdose (≥50 mg/kg administered into the carotid artery catheter). Power analysis based on a known sample variability of PO_2mv and anticipated supplementation effects [22,25] indicate that six rats per group would be sufficient to demonstrate a statistical difference, if present.

2.5. PO_2mv measurement and curve-fitting

The Stern–Volmer relationship allows the calculation of PO_2mv through the direct measurement of a phosphorescence lifetime via the following equation [30]:

$$PO_{2mv} = [(\tau^\circ/\tau) - 1]/(k_Q \times \tau^\circ)$$

where k_Q is the quenching constant and τ° and τ are the phosphorescence lifetimes in the absence of O_2 and the ambient O_2 concentration, respectively. For G2, k_Q is 273 mmHg/s and τ° is 251 μs at 38 °C [31] and these characteristics do not change over the physiological range of pH and temperature in the rat in vivo and, therefore, the phosphorescence lifetime is determined directly by the O_2 pressure [30,31].

Curve-fitting of the measured PO_2mv responses was performed with commercially available software (SigmaPlot 11.01, Systat